These inhibitors were discovered by targeting *in silico* the switch, and, interestingly, similar compounds were shown to target the switch region of bacterial RNAP (Sahner, 2013; Fruth, 2014). Beside, interactions between adefovir compound and EF have been extensively studied (Shen, 2004; Česnek, 2018). An X-ray crystallographic structure of the EF/adefovir complex displays the compound bound into the catalytic site in the presence of a Rubidium ion. The disordered switch observed in the inactive EF and the partial knowledge of the interactions between the protein and these inhibitors open a wide range of possibilities for the interactions. In the present work, we use various experimental approaches, biochemical assays and NMR, as well as molecular modeling to investigate these interactions.

Česnek et al. Analogues as Potent and Selective Inhibitors of Adenylate Cyclases from *Bordetella pertussis* and *Bacillus anthracis*. ChemMedChem 2018.
Fruth et al. Binding mode characterization of novel RNA polymerase inhibitors using a combined biochemical and NMR approach. ACS Chem Biol 2014.
Laine et al. Use of allostery to identify inhibitors of calmodulin induced activation of *Bacillus anthracis* edema factor. Proc Natl Acad Sci USA 2010.
Sahner et al. Novel small molecule inhibitors targeting the "switch region" of bacterial RNAP: structure-based optimization of a virtual screening hit. Eur J Med Chem 2013.
Shen et al. Selective inhibition of anthrax edema factor by adefovir, a drug for chronic hepatitis B virus infection.Proc Natl Acad Sci USA 2004.

2392-Pos

Study on the Mechanism of Anti C-Met Activity of Boc-Protected Amino Groups of Bithiazolophanes by using SILCS

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We have synthesized some bithiazolophane derivatives and analyzed inhibitory activity against protein kinases, including c-Mets. Only 2,15-Bis(*tert*-butoxycarbonyl)-2,15-diaza[3.3](2,2')(4,4'-bithiazolophane) (BOCBTP) showed anti-c-Met activity (IC50 = 603 nm) among the bithiazolophane analogs. When we changed the *tert*-butoxy groups in this compound into *iso*-butoxy groups, the anti-c-Met activity of this compound diminished. To understand why only BOCBTP showed anti-c-Met activity, we applied a computational simulation method: Site-Identification by Ligand Competitive Saturation (SILCS) and c-Mets X-ray crystal structures with bound ligands. Characterization and identification of the binding site of our compounds was performed using docking simulation in SILCS.

Based on X-ray crystal structures one main active site in c-Mets was identified, which can accept two types of ligands, bent or long straight ligands. Both these ligands have the following interactions; hydrophobic interaction, hydrogen bond and π-π stacking. As SILCS simulation also showed the hydrophobic and some hydrophilic areas in the same site, the results confirmed the active site of c-Met. Our compounds were also placed at a possible alternative binding site identified by SILCS. When our compounds were docked into the site, only one side chain placed there and basic skeleton was out of the pocket. Although BOCBTP also placed at same position, BOC groups of BOCBTP located near aspartic acid residues. If two BOC groups will be cleaved with protons of aspartic acid, the steric shape of BOCBTP would change into a flat shape. As the flat shape of BOCBTP derivatives can enter into the active site deeply, it is hypothesized that this is the reason only BOCBTP showed anti-c-Mets activity.

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Unveiling the Role of Surfactants on Amyloid-Like Protein Self-Assembling

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Some proteins can undergo structural changes that may trigger an aggregation process where they self-assembly into highly ordered aggregates called amyloid fibers. In vivo, these amyloid fibers are related to more than 25 different diseases, some of them are lethal as Creutzfeldt-Jakob disease and others can lead a person to incapacities, as diabetes type II, Alzheimer's and Parkinson's diseases. With the aim of understanding the conditions and mechanisms by which proteins form amyloid fibers, we mixed bovine serum albumin (BSA) at pH 3.7 with sodium dodecyl sulfate (SDS) and sodium perfluorooctanoate (SPFO) to induce the amyloid fibers formation. BSA conformational changes were followed in order to suggest a possible pathway of aggregation. Dynamic Light Scattering (DLS) and Thioflavin T fluorescence data revealed, respectively, the presence of large aggregates and the formation of amyloid-like fibers as the surfactant concentration increased, whereas circular dichroism (CD) showed that BSA second structure changes from α-helix to β-sheet. Transmission electron microscopy (TEM) permitted us to obtain images of fibers and aggregates in the micrometers scale. Further, small angle x-ray scattering (SAXS) measurements provided information about the protein's quaternary structure as a function of surfactant concentration and a more detailed analysis allowed us to suggest a pathway of fibrillation process. Lastly, we performed molecular dynamics (MD) simulations to obtain an all atom structure of BSA at pH 3.7 to study in the molecular level the different effects of SDS or SPFO in the BSA conformation and we also used the MD structures to modeling the SAXS experimental curve.

2394-Pos

Nuclear Magnetic Resonance at the Interface: Identifying Preferred Binding Regions in Multimodal Cation Exchange Chromatography using Functionalized Nanoparticles

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The Fc domain is an important component of complex molecules such as monoclonal antibodies (mAb) based biologics as well as fusion proteins and is highly conserved across a given class of mAbs. Recent work in our lab has shown the importance of specific domains towards binding for mAbs to multimodal (MM) cation exchange chromatographic systems. In this collaborative work, we employ a combination of Nuclear Magnetic Resonance (NMR) spectroscopy and Umbrella sampling simulations to develop a fundamental understanding of how multimodal ligands and surfaces interact with the Fc domain. We performed NMR titration experiments with isotopically labeled Fc and ~15 nm diameter gold nanoparticles (Au NPs) functionalized with Self Assembled Monolayers (SAMs) presenting different MM ligands at different ligand densities. The results showed the interface of C_H2 and C_H3 domains and the hinge region to be preferred binding regions for interaction of Fc to MM ligand surfaces. These flexible regions on the protein surface which are rich in positive and aliphatic residues are important for the binding of Fc to MM ligand surfaces. Further, to shed light on the binding mechanisms, we performed umbrella sampling with the Fc held at key orientations above a SAM surface presenting MM ligands at relevant ligand densities similar to those tested experimentally. These simulations suggested that as the Fc was brought close to the surface, the residues near the hinge region first made contact with the surface followed by a tighter binding facilitated by interaction of His, Val, Leu and Ile residues at the interface of the C_H2 and C_H3 domains. This combined experimental and simulations approach enabled us to develop a molecular level understanding of the binding for complex biological products in MM CEX systems.

2395-Pos

How does Glycosylation Affect Drug Binding on Influenza? The Roles of Electrostatics and Sterics Examined through Brownian Dynamics Simulations

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Influenza is one of the most well-known viruses, yet much remains to be discovered. Glycans, the ubiquitous polysaccharides that decorate the surface of influenza membrane proteins, play a complex part in influenza's life functions. They are known to modulate influenza's transmissibility, virulence, electrostatics, sterics, cooperativity, drug binding, dynamics, antibody response, and immune evasion, amongst other aspects, but their exact role is not always well-understood. Additionally, experimental influenza work is challenging to deconvolute due to complex and overlapping interactions, and due to influenza's mutability. These difficulties can be bypassed by creating these systems computationally. To examine how glycan and influenza strain differences affect the binding kinetics of Tamiflu and Relenza, we created *in silico* systems of neuraminidase from avian influenza (H5N1 A/Vietnam/1203/2004) and swine flu (H1N1 A/California/04/2009) with experimentally-derived, biologically-relevant glycoprofiles. We then benchmarked association rates of the drugs to the active site of neuraminidase with Brownian dynamics, a type of simulation evaluating the electrostatics and sterics of a system. Next, we modulated this association rate by removing glycans, to deconvolute the role of electrostatics and sterics in drug association to neuraminidase, and how this varies across strain and glycoprofile. These results increase our understanding of how the viral glycoprofile influences drug association, and has implications for vaccine optimization.